ESTIMATION OF GENOME SIZE (C-VALUE) IN IRIDACEAE BY CYTOPHOTOMETRY[1]

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ABSTRACT

Nuclear genome sizes have been calculated for 19 genera and 30 species of Iridaceae using cytospectrophotometry. Mean extinction values for nuclei in squashes of actively growing root tips stained in Schiff's reagent were compared with a standard, maize, of known genome size, treated in the same way. Values range from lows of 1.1 to 4.9 pg DNA per nucleus in diploid species of subfamily Ixioideae to a high of 65.1 pg in *Iris histrio,* subfamily Iridoideae. Genome size in diploid Iridoideae ranges from a low in *Galaxia fugacissima* of 6.4 pg to the ten fold high observed in *I. histrio.* Results are, in general, consistent with what is known about cytology and phylogenetic relationships of the taxa studied. Polyploid species have close to twice the values obtained in closely related diploids, and allied species or genera have very similar genome sizes.

Iridaceae are a plant family of nearly worldwide distribution, comprising some 1,500 species in about 85 genera usually assigned to two or three subfamilies, Iridoideae, from which Sisyrinchioideae may not be separable, and Ixioideae. Species are concentrated in Africa where more than half the genera and species occur, and in South and Central America including Mexico. The systematics of the family is comparatively well known, particularly in the Old World. Chromosome cytology is also well known, and unusually varied for a family of this size. Chromosome size ranges from very small in some Australasian and South American genera to very large in Old World genera such as *Iris, Moraea,* and their allies, while base numbers for genera range from $x = 16$ to 6. Chromosome numbers and karyotypes are known for most genera and for many species in most of these but there have not been until now any satisfactory measurements of absolute size of the genome of various genera and species, i.e., the amount of DNA per cell or C-value. In this paper we present measurements of nuclear genome size for a wide range of species and genera of Iridaceae following a standard cytospectrophotometric method for estimating DNA content. Genome sizes for the 30 species in 19 genera studied here have been calculated by comparison against a standard, *Zea mays,* of known genome size, 6.3 pg (Hake & Walbot, 1980).

MATERIALS AND METHODS

Plants studied were all of wild origin, the collection data and voucher information for which is presented in Table 1. Measurements were made on nuclei in root tip apices fixed in Carnoy's 3:1 absolute ethanol:glacial acetic acid and stained in Schiff's reagent. Root tips of the maize standard were fixed and stained at the same time and in the same way as the species of Iridaceae. Cytophotometric determinations were made using a Zeiss Universal microscope equipped with a Zeiss Type 03 Microphotometer with an automatic scanning stage. A planapochromat oil immersion objective NA 1.32 × 100 was used for all measurements.

Approximately 20 measurements were made for each species. Mean relative values of the amount of DNA per cell were calculated for each species by obtaining the average of the lower readings (2C-values) and half the high readings (4C-values). Low readings represent cells in a post mitotic phase before the onset of duplication of the genome and the high readings represent cells that have completed the duplication of the

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TABLE 1. Voucher information for the species used in this study. All specimens are housed at Missouri Botanical Garden (MO).

Species	Collection Data
SUBFAMILY IRIDOIDEAE (Including SISYRINCHIOIDEAE)	
OLD WORLD TAXA	
Iris histrio L.	Israel, Golan Heights, *Goldblatt s.n.*, no voucher
Dietes grandiflora N. E. Br.	S. Africa, Cape, Riebeek East, *Bayliss 7014*
Galaxia fugacissima (L. f.) Druce	S. Africa, Cape, Middleton, Caledon, *Goldblatt 2631*
Moraea anomala Lewis	S. Africa, Cape, Elim, *Goldblatt 2616*
M. inconspicua Goldbl.	S. Africa, Cape, Botrivier-Hawston, *Goldblatt 3300*
M. ciliata (L. f.) Ker	S. Africa, Cape, Wolseley, *Goldblatt s.n.*, no voucher
M. fugax (de la Roche) Jacq.	
population 1	S. Africa, Cape, Klawer, *Goldblatt 5778*
population 2	S. Africa, Cape, Koeberg, *Goldblatt 4080*
M. atropunctata Goldbl.	S. Africa, Cape, Caledon distr., *Goldblatt 5635*
M. calcicola Goldbl.	S. Africa, Cape, Saldanha hills, *Goldblatt 4118*
M. tulbaghensis L. Bol.	S. Africa, Cape, Gouda, *Goldblatt s.n.*, no voucher
M. villosa (Ker) Ker	S. Africa, Cape, below Gydo Pass, *Goldblatt 2594*
M. unguiculata Ker	S. Africa, Cape, Steinkopf, *Goldblatt 2777*
M. bipartita L. Bol.	S. Africa, Cape, near Hankey, *Goldblatt 2076*
Hexaglottis namaquana Goldbl. ined.	S. Africa, Cape, Spektakelberg, Namaqualand, *Goldblatt 3059*
Homeria bifida L. Bol.	S. Africa, Cape, Rebunie, Calvinia, *Goldblatt 3894*
H. pendula Goldbl.	S. Africa, Cape, Kamiesberg, *Goldblatt 4306*
H. flaccida Sweet	S. Africa, Cape, Twenty Four Rivers, *Goldblatt 3924*
Sessilistigma radians Goldbl. ined.	S. Africa, Cape, near Macgregor, *Goldblatt 5903*
Gynandriris setifolia (L. f.) Foster	S. Africa, Cape, near Matjesfontein, *Goldblatt 3215*
Roggeveldia fistulosa Goldbl.	S. Africa, Cape, Roggeveld Escarpment, *Goldblatt 4163*
NEW WORLD TAXA	
Cipura paludosa Aubl.	Nicaragua, *Henrich 143*
Sisyrinchium convolutum Nocca	Nicaragua, *Henrich 152*
SUBFAMILY IXIOIDEAE	
Anomatheca viridis (Ait.) Goldbl.	S. Africa, Cape, Olifantskop, Langebaan, *Goldblatt 2335*
Freesia alba (G. L. Meyer) Gumbleton	S. Africa, Cape, Sandbaai, Hermanus, *Goldblatt 5293*
Watsonia brevifolia Ker	S. Africa, Cape, near Albertinia, *Goldblatt 4855*
Pillansia templemanii L. Bol.	S. Africa, Cape, Arieskraal, Caledon dist., *Powrie s.n.*
Lapeirousia verecunda Goldbl.	S. Africa, Cape, Spektakelberg, Namaqualand, *Goldblatt 2789*
Gladiolus virescens Thunb.	S. Africa, Cape, near Botrivier, *Goldblatt 5641*
Hesperantha bachmannii Baker	S. Africa, Cape, Wildepaardehoek, Namaqualand, *Goldblatt 5754*
Babiana virginea Goldbl.	S. Africa, Cape, Roggeveld, *Goldblatt s.n.*

genome but have not begun to divide. The high readings were consistently approximately twice the readings in the low range. Intermediate readings were disregarded. In all samples studied, readings showed a two-fold low to high range. Difficulty was experienced in obtaining measurements in Ixioideae. The nuclei were weakly stained and contrasted poorly with the background cytoplasm. Genome size in this subfamily is undoubtedly very low in comparison to maize and to the other species of Iridaceae studied. The percent error in our measurements for this group is very high, but the results are, nevertheless, of value for comparison with other Iridaceae.

RESULTS AND DISCUSSION

The results of our measurements of genome size relative to the maize standard are reported in Table 2, along with the standard deviation of the measurement. The haploid and basic chromosome number of each species, also included in the table, are taken from previously published accounts (Goldblatt, 1971, 1976, 1979, 1980) or

from papers in preparation. This represents the only extensive set of measurements in Iridaceae of nuclear genome size, which, in non green cells, can be regarded as essentially equivalent to the total cellular DNA content. The only previous determinations of genome size in Iridaceae, according to Bennett's (1972) review of amounts of nuclear DNA in angiosperms, are two reports for *Gladiolus*. One, by Sparrow et al. (1965), for a cultivar, *Gladiolus* 'Friendship,' is an estimated value of 13.5 pg. In the other, Baetke et al. (1967) obtained 6 pg for *Gladiolus* 'Mansoor.' In both cases the material studied was reported to be tetraploid. This second report is in fairly close agreement with our own estimation of 3.2 pg for a diploid species of this genus. The much higher figure reported by Sparrow et al. must apparently be disregarded. The relatively low genome sizes established for *Gladiolus* seem characteristic for subfamily Ixioideae, in which a range of values from 1.1 to 4.9 pg have been determined for seven diploid species, each a different genus. The genome size in the tetraploid *Pillansia*, 5.4 pg, is also consistent with the range for Ixioideae. These results are consistent with karyotypic observations for Ixioideae (Goldblatt, 1971), in which small chromosomes are characteristic and there seems no substantial variation in the total amount of chromosome material, as estimated by linear chromosome measurement, in diploid members of a range of genera of this subfamily, from *Babiana* with a low $x = 7$ to *Gladiolus* with $x = 15$.

Subfamily Iridoideae provides a sharp contrast. Genome sizes range from 6.4 pg in *Galaxia fugacissima* to a high of 36.4 pg in *Moraea calcicola*, among diploid species in Southern Africa. A 65.1 pg genome was found in the Middle Eastern *Iris histrio*, a species possibly of tetraploid origin although it is not polyploid compared with closely related species. The figures reflect the large difference in total chromosome size of Old World Iridoideae vs. Ixioideae, pointed out by Goldblatt (1971). The results also seem to confirm Goldblatt's (1976) contention that cytological evolution in *Moraea*, diploid species of which have haploid numbers of $n = 10, 9, 8, 7, 6$, and 5, proceeded from a basic chromosome number of $x = 10$ to a derived $x = 5$ by aneuploid decrease. Representatives of the three primitive subgenera of *Moraea*, *M. ciliata* (subgenus *Moraea*), *M. inconspicua* (subgenus *Visciramosa*), and *M. anomala* (subgenus *Monocephalae*), all $x = 10$, have genome sizes of 22 to 23 pg. Species of the derived subgenus *Vieusseuxia*, all $x = 6$, have genomes ranging from 23.4 pg in *M. bipartita* to 33.1 in *M. atropunctata* and 36.4 pg in *M. calcicola*. Two tetraploid species of *Moraea*, *M. villosa*, and *M. tulbaghensis*, have genomes of 72.4 pg of DNA, a value remarkably close to twice the 36.4 pg value obtained for the nearly allied *M. calcicola*.

There is some evidence in *Moraea* subgenus *Vieusseuxia* of the C-value paradox (Walbot & Goldberg, 1979). Related species of the same subgenus and section, and with similar karyotypes have unexpected differences in genome size (Table 2). The difference between the genome size of *M. unguiculata* and either *M. atropunctata* or *M. calcicola* is of the order of 50%. In genera allied to *Moraea* such as *Homeria* this paradox is also evident. In *Homeria*, most species of which have a basic $x = 6$ and very similar karyotype, *H. pendula* has a genome size of 22.5 pg, while *H. bifida* has 29.2 pg and the tetraploid *H. flaccida*, 41.2 pg. *Sessilistigma*, an undescribed monotypic genus closely related to *Homeria*, has a genome size of 31.6 pg, a figure consistent with the range found here in *Homeria*.

Hexaglottis, a genus also probably allied to *Homeria* has a genome size of 20.6 pg. This is low in comparison with *Homeria* but consistent with cytological observations which indicate a chromosome complement very similar, but slightly smaller than in *Homeria*. In examples of two other genera of Iridoideae, *Gynandriris* has a genome size of 24.1 pg, and *Roggeveldia* has 16.5 pg. Of the species studied here, these two genera are probably most closely related to *Moraea bipartita*, the genome size of which is 23.4 pg. The genome size data tend to support the hypothesis that there is a reasonably close relationship between *M. bipartita* and *Gynandriris*, their genomes being very similar in size. *Roggeveldia*, which seems related to this group (Goldblatt, 1979), presumably has lost a substantial amount of DNA in the course of its evolution if its relationships are, in fact, with these species.

Of the two New World taxa examined, *Sisyrinchium convolutum* (an octoploid with $n = 36$) has a genome size of 10.9 pg and *Cipura paludosa* (a tetraploid form with $n = 14$) has 19.5 pg. Basic genome size in *Sisyrinchium* would accordingly be of the order of 2.7 pg. This is comparable with Ixioideae rather than with Iridoideae, to which *Sisyrinchium* is usually allied. *Cipura* on the other hand has a basic genome size of the order of

TABLE 2. Mean genome size (C-value) for 30 species of Iridaceae with standard deviation (s.d.), haploid chromosome number (*n*) and basic chromosome number (*x*), arranged by subfamily.

Species	C-value	s.d.	*n*	*x*
SUBFAMILY IRIDOIDEAE (Including SISYRINCHIOIDEAE)				
OLD WORLD TAXA				
Iris histrio L.	65.1	±1.6	10	10
Dietes grandiflora N. E. Br.	13.5	0.2	10	10
Galaxia fugacissima (L. f.) Druce	6.4	0.8	9	9
Moraea anomala Lewis	22.0	1.3	10	10
M. inconspicua Goldbl.	23.0	1.3	10	10
M. ciliata (L. f.) Ker	22.7	0.9	10	10
M. fugax (de la Roche) Jacq.				
population 1	19.9	1.2	6	10
population 2	19.9	0.7	6	10
M. atropunctata Goldbl.	33.1	1.9	6	6
M. calcicola Goldbl.	36.4	2.5	6	6
M. tulbaghensis L. Bol.	72.4	3.4	12	6
M. villosa (Ker) Ker	72.4	1.3	12	6
M. unguiculata Ker	26.2	3.4	6	6
M. bipartita L. Bol.	23.4	0.6	6	6
Gynandriris setifolia (L. f.) Foster	24.1	1.2	6	6
Roggeveldia fistulosa Goldbl.	16.5	0.3	6	6
Hexaglottis namaquana Goldbl. ined.	20.6	0.7	6	6
Homeria bifida L. Bol.	29.2	1.7	6	6
H. pendula Goldbl.	22.5	1.6	6	6
H. flaccida Sweet	41.2	2.5	12	6
Sessilistigma radians Goldbl. ined.	31.6	1.9	6	6
NEW WORLD TAXA				
Cipura paludosa Aubl.	19.5	1.4	14	7
Sisyrinchium convolutum Nocca	10.9	0.6	36	9
SUBFAMILY IXIOIDEAE				
Anomatheca viridis (Ait.) Goldbl.	1.9	0.9	11	11
Freesia alba (G. L. Meyer) Gumbleton	3.7	0.3	11	11
Watsonia brevifolia Ker	1.6	0.5	9	9
Pillansia templemanii L. Bol.	5.4	2.2	20	10
Lapeirousia verecunda Goldbl.	4.9	0.5	8	10
Gladiolus virescens Thunb.	3.2	0.3	15	15
Hesperantha bachmannii Baker	1.1	0.3	13	13
Babiana virginea Goldbl.	3.5	0.4	7	7

9.8 pg, much larger than *Sisyrinchium,* and a difference clearly reflected in the karyology (Goldblatt, 1981). The genome size accords well with the Old World Iridoideae, although it is somewhat on the small side for the subfamily, and there is no doubt that *Cipura* is a member of Iridoideae.

The reasons for the often large differences in genome size among genera and species of Iridaceae are obscure. All members of the family are, with minor exceptions, long lived, geophytic perennials so that differences in life cycle (Bennett, 1972) cannot be used to explain genome size differences. In particular, the Old World taxa studied here all have a similar life cycle and similar environmental and edaphic requirements. The reasons for the primary difference in the genome size between Iridoideae and Ixioideae thus appear rooted in the evolutionary history of these subfamilies. The secondary differences

within Iridoideae, between closely allied genera and within genera, apart from polyploidy, are equally difficult to explain and we can offer no reasonable explanation for the genomic differences in the taxa studied here. It is clear that if the primitive genus *Dietes* (Goldblatt, 1981) is regarded as having close to the basic genome for Iridoideae, then trends for both a decrease (in *Galaxia*) or an increase (in *Moraea, Homeria,* etc.) in genome size have taken place during the evolution of the subfamily.

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